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Effects of Dietary *Allium hookeri* Root Powder on the Body Fat Deposition and Biochemical Parameters in Guinea Pigs

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Abstract

The overweight and obesity caused and exacerbated many diseases. Therefore, consumer demands for functional food which can prevent or decrease overweight and obesity is increased. This study was performed to investigate the effects of the dietary *Allium hookeri* root powder on the body fat deposition and biochemical parameters in guinea pigs. The guinea pigs were fed aF control diet (CON), as well as the control diet including 1% freeze-dried *Allium hookeri* root powder (AH). The final body weight, total body and weight gain, total feed intake and feed efficiency ratio (FER) were not significantly different between CON and AH groups ($p<0.05$). However, the weight of perirenal adipose tissue (PAT) and the epididymal adipose tissue (EAT) in the AH were significantly lower than those of the CON ($p<0.05$). The triglycerides, total cholesterol and low-density lipoprotein (LDL) cholesterol levels in the AH were significantly lower than those of the CON ($p<0.05$). The saturated fatty acid (SFA) levels in the AH were significantly lower than those of the CON ($p<0.05$). The results of this study suggested that the dietary *Allium hookeri* root powder may decrease fat accumulation, and to improve blood hyperlipidemia in guinea pigs.

Keywords: *Allium hookeri*; Foot powder; Fat deposition; Triglycerides; LDL cholesterol; Free fatty acids

Introduction

The radical development of society has been increasing the necessity and the importance of health [1]. Before the emergence of health issues, consumers consumed foods only to fulfill their appetite, but recently consumers have started to consume foods which improve health or decrease the possibility of developing particular diseases [2]. Many diseases such as cardiovascular disease, hypertension and diabetes are caused or exacerbated by obesity, so many studies have investigated dietary supplementation for the prevention of obesity [3,4].

Some functional foods have been approved for obesity prevention [3]. Functional foods such as plants rich in phenolic compounds and organic sulfur compounds help to decrease metabolic syndrome by reducing cholesterol, body weight, and triglycerides [5]. Plant extracts, or minerals such as sulfur, can affect metabolism, and are used for maintaining health [6,7].

Sulfur is a mineral that has been associated with improvement of cardiovascular functions, as well as being an anti-oxidant; it also has anti-obesity abilities, has been used in oriental medicine for neural paralysis and cold extremity treatment, and in western medicine to treat constipation, hemorrhoids, and to support homeostasis [8,9]. However, due to its high toxicity, detoxifying process is necessary for it to be used as a dietary supplement [10].

There are several methods of purification [11], such processing the sulfur by heating and melting it over a material or light mineral, and then separating the liquid sulfur from the cooled material. Recently, studies were done on animals that were fed processed sulfur or organic sulfur compounds, to reveal the positive effect of sulfur on the quality of meat and its health [12,13].

Allium hookeri, belonging to the *Allium* genus (which includes plants such as garlic, onions, and chives), has been shown to improve the nutritional quality of food [14]. It is used for medical purposes, because of its anticancer and anti-inflammatory properties, and is listed in the medical dictionary of Myanmar [15].

Plants rich in organic sulfur compounds and phytosterol have antioxidant, anticancer, anti-inflammatory and LDL cholesterol-lowering effects [16] *Allium hookeri* has six times higher than the organic sulfur compound content than garlic, and two times higher than the content of onions cultivated in Korea [17].

Allium hookeri started successfully cultivated in Korea [18]. There are many studies which document the effects of the dietary supplementation of Allium species (such as garlic and onions) but few studies on *Allium hookeri* have been done. Therefore, the aim of this study was to investigate the effect of

the dietary *Allium hookeri* root powder on the fat deposition and biochemical parameters in guinea pigs.

Materials and Methods

Composition of diet

Allium hookeri root was purchased from Samchaenara Co. (Gyeongsangnamdo, Korea) and freeze-dried at Dongil Cold Storage & Foods Co. (Gyunggido, Korea). The freeze-dried *Allium hookeri* root powder used in this study contained 12.72 ± 0.79% crude proteins, 0.46 ± 0.06% crude fat, 31.67 ± 0.12% crude fiber, 1.60 ± 0.16% moisture and 12.24 ± 2.24% crude ash and contained 7,382 mg/kg of the organic sulfur compounds. The proximate composition of freeze-dried *Allium hookeri* root powder measured by according to the AOAC method [19].

The crude protein was determined using the Kjeldahl method; the crude fat was evaluated using the Soxhlet method, and the crude fiber was measured using the Henneberg-Stohmann methods. The moisture content was evaluated by drying in a 105°C oven, and the crude ash was measured at 450°C in burning using the drying method.

Animal experimental design

Ten 6-week old conventional Hartley male guinea pigs weighing between 250 g and 300 g (RaonBio Co., Gyunggido, Korea) were used. During the acclimation period, the guinea pigs were fed cabbage and control diets at the ratio of 5:5, which was gradually reduced by removing the cabbage and introducing the control diets [20].

After a week of acclimation, they were randomly divided into two groups and placed in individual cages. The control group (CON) was fed the control diet. The AH group was fed the control diet, plus 1% *Allium hookeri* root powder. The composition of the diets is presented in **Table 1**.

The animals received free access to clean water containing ascorbic acid (500 mg/L) [21]. All guinea pigs were maintained under a 12 hour light and dark cycle at a controlled room temperature of 22 ± 2°C, with moisture levels of 50 ± 5%.

The experiment was conducted over an 8-week period. The animal experiment was approved by the Konkuk University Institutional Animal Care and Use Committee (IACUC, Approval No. KU14025).

Table 1: The composition of the diets.

Ingredients (g/kg diet)	Groups	
	CON[1]	AH
Casein, 30 Mesh	200	198
L-Cystine	3	3
Corn Starch	397	393
Maltodextrin 10	132	131
Sucrose	100	99
Cellulose	50	50
Soybean Oil	70	69
Mineral Mix2	35	5
Vitamin Mix2	10	10
Choline Bitartrate	3	3
Allium hookeri root powder	-	10
Total (g)	1,000	1,000
Total (kcal)	4,000	4,000

[1]Con: Guinea pigs fed the control diet; AH: Guinea pigs fed control diet including 1% *Allium hookeri* root powder. 2AIN 93G. (Research Diets, INC., New Brunswick, NJ, USA).

Initial and final weight, weight gain, total food intake and feed efficiency ratio

The guinea pigs were weighed before they were divided into random groups, and the final body weight of the guinepigs was checked before fasting for 24 hours, prior to dissection. Food intake and changes in body weight were measured at the same time every day. The guinea pigs were weighed using an electronic balance (AR2140, Ohaus, Pine Brook, NJ). The feed efficiency ratio (FER) was calculated using the formula below.

Total food intake (g) = ration of food (g) - remaining food (g)

$$\mathrm{FER} = \frac{\text{Total body weight gain (g)}}{\text{Total food intake (g)}}$$

Organs and adipose tissue weight

The liver, spleen, kidney, heart, perirenal adipose tissue (PAT) and epididymal adipose tissue (EAT) were removed immediately after sacrificing, and weighed after being cleaned with 95% saline solution. The organ weight was presented as the organ to body weight ratio (g/kg body weight) [22]. The organs were weighed using an electronic balance (AR2140, Ohaus, Pine Brook, NJ).

Blood analysis

The blood was collected with a sterilized syringe after dissection. The blood was left at room temperature for 3 hours and then centrifuged at 5,000 rpm for 20 min at 4°C to separate the serum. Before analysis, the blood was frozen in a refrigerator set below -20°C. The triglycerides, total cholesterol, high-density lipoprotein (HDL) -cholesterol and low-density lipoprotein (LDL) -cholesterol were determined using a chemistry analyzer ADIVA 1650 (Bayer, New York, NY, USA) with assay reagents (Bayer). LDL-cholesterol was calculated with the Friedewald formula. Measurement of amino acids and fatty acids was performed by the Korean Standards Codex method [23]. After the preconditioning procedure, blood samples were analyzed by an automatic amino acids analyzer (S2100, S4300, S5200, SYKAM, Darmstadt Germany) to measure amino acid content. The free fatty acids were measured using a gas chromatograph (5890 GC, Agilent Technologies, Santa Clara, CA, USA) with a flame ionization detector SP-2560 column (100 m × 0.25 mm × 0.2 µm) (Signa-Aldrich, USA), after preconditioning.

Statistical analysis

A statistical analysis was performed using SPSS software (SPSS Inc., Chicago, IL, USA). All data were analyzed using the General Linear Models (GLM). The results of statistical analysis were shown as mean and the standard error of means (SEM). Significant differences were determined using the ANOVA (one way analysis of variance). Statistical differences were considered significant at $p<0.05$.

Results and Discussion

Growth performances

The final body weight, body weight gain and FER can be affected by dietary supplement. The growth performances are provided in **Table 2**. The final weight, body weight gain, total food intake, and the FER of the AH was not significantly different from the CON ($p<0.05$). Previous studies reported the decreased body weight of processed sulfur-fed rats [8,9]. However, there is a previous report that garlic (*Allium sativum*) increased body weight in broiler chickens [24]. However, there was no report that *Allium hookeri* effect on the body weight. This experiment suggested that *Allium hookeri* root powder did not affect the final body weight, body weight gain, total food intake and FER of the guinea pigs.

Table 2: The effect of dietary supplement on the growth performances of guinea pigs.

Composition	CON[1]	AH	SEM	P-value
Body weight (g)				
Initial	268.4	261.6	9	0.608
Final	433.6	423	17.34	0.678
Body weight gain (g)	165.2	161.4	10.01	0.795
Total food intake (g)	716.5	729.5	23.94	0.711
FER	0.23	0.2	0.02	0.314

[1]Con: Guinea pigs fed the control diet; AH: Guinea pigs fed control diet including 1% *Allium hookeri* root powder; SEM: Standard Error of Means; FER: Feed Efficiency Ratio. The statistically different ($p<0.05$) is written as $P<0.001^{***}$; $P<0.01^{**}$; $P<0.05^{*}$ (n=5).

Organs and adipose tissue weight

The organ weight and adipose tissue weights were presented in **Table 3**. The weight of the liver, kidney, spleen and heart were in the AH group were not significantly different from the CON ($p<0.05$). A previous study also reported that differences in sulfur concentration and did not affect the weight of organs in rats [25].

The results of the organs weight in this study were in agreement with previous studies, so this study suggested that diets supplemented by *Allium hookeri* root powder may not directly affect the weight of the organs in guinea pigs. However, the PAT and EAT weight of the AH was significantly lower compared to that of the CON ($p<0.05$).

Previous study reported that a plants rich in phenolic compounds and organic sulfur compounds help to decrease metabolic syndrome [5]. This result may suggest that even though there was no significant difference in final weight, body weight gain, total food intake, and FER between CON and AH groups, dietary *Allium hookeri* root powder affect the adipose tissue deposition in guinea pigs.

There is also previous research reported that processed sulfur-fed chickens had an increase of abdominal fat, but a decrease in the total body fat, because supplementing with sulfur accelerated the lipid metabolism in the chickens [24].

This result may suggest that *Allium hookeri* root powder may decrease the adipose tissue by accelerating the lipid metabolism, although it did not affect the body weight.

Table 3: The effect of dietary supplement on the organs and adipose tissues weights of guinea pig.

Items (g/kg body weight)	CON[1]	AH	SEM	P-value
Liver	40	34	2.64	0.217
Kidney	8.2	7.43	0.34	0.147
Spleen	1.73	1.23	0.16	0.054

Heart	3.43	3.32	0.18	0.657
PAT	5.06	2.4	0.75	0.037*
EAT	4.27	3.48	0.13	0.002**

[1]Con: Guinea pigs fed the control diet; AH: Guinea pigs fed control diet including 1% *Allium hookeri* root powder; SEM: Standard Error of Means; PAT: Perirenal Adipose Tissue; EAT: Epididymal Adipose Tissue. The statistically different ($p<0.05$) is written as $P<0.001^{***}$; $P<0.01^{**}$; $P<0.05^{*}$ (n=5).

Triglyceride and cholesterol levels in serum

The high triglyceride levels in serum are significant risk factor for the cardiovascular disease and associated with body weight [26]. The triglyceride and total cholesterol, HDL-cholesterol, LDL-cholesterol results are shown in **Table 4**. The serum triglyceride contents in the *Allium hookeri* root power treated group was significantly decreased than in the CON ($p<0.05$). The levels of total cholesterol and LDL-cholesterol in the AH group was significantly lower than the CON group ($p<0.05$). The consumption of processed sulfur increased both the triglyceride and cholesterol levels in blood plasma, which was also reported previously [10,11]. However, the consumption of foods rich in organic sulfur compounds and phytosterols also reported a cholesterol-lowering effect [26,27]. Also, there was a previous study shown that *Allium hookeri* lowered the serum LDL cholesterol [17]. Lowering LDL cholesterols is rink to decrease the risk of cardiovascular disease [28]. This study also indicated that a dietary supplement of *Allium hookeri* root powder can lower serum cholesterol levels, which is in agreement with previous reports.

Table 4: The effect of dietary supplement on the triglyceride, total cholesterol, HDL cholesterol, LDL cholesterol analysis of guinea pig.

Items (mg/dL)	CON[1]	AH	SEM	P-value
Triglyceride	104.67	72.67	2.19	0.027**
Total cholesterol	66.33	61	1.11	0.000***
LDL-cholesterol	61.33	47.33	3.18	0.036**
HDL-cholesterol	7.33	6.67	0.67	0.519

[1]Con: Guinea pigs fed the diet; AH: Guinea pigs fed control diet including 1% *Allium hookeri* root powder; SEM: Standard Error of Means; LDL: Low-Density Lipoprotein; HDL: High-Density Lipoprotein. The statistically different ($p<0.05$) is written as $P<0.001^{***}$; $P<0.01^{**}$; $P<0.05^{*}$ (n=3).

Free fatty acids composition

Analysis of the free fatty acids in the serum is shown in **Table 5**. Total saturated fatty acids (SFA) contents in AH showed significantly lower levels than that of the CON ($p<0.05$). A previous study reported higher levels of SFA and lower levels of monounsaturated fatty acids (MUFA) in obese rats [29]. This result may suggest that consuming *Allium hookeri* root power decreased the total SFA.

Table 5: The effect of dietary supplement on the free fatty acids contents of guinea pig.

Items(mg/dL)	CON[1]	AH	SEM	P-value
Total SFA	3.86	2.51	0.25	0.018*
Total MUFA	56.53	57.24	3.97	0.906
Total PUFA	7.73	7.77	1.21	0.781
Total PUFA/SFA	1.85	3.47	0.72	0.186

[1]Con: Guinea pigs fed the control diet; AH: Guinea pigs fed control diet including 1% *Allium hookeri* root powder; SEM: Standard Error of Means; SFA: Saturated Fatty Acid; MUFA: Mono Unsaturated Fatty Acid; PUFA: Poly Unsaturated Fatty Acid. The statistically different ($p<0.05$) is written as $P<0.001^{***}$; $P<0.01^{**}$; $P<0.05^{*}$ (n=5).

Amino acids composition

Analysis of amino acids in the serum is provided in **Table 6**. The amino acids of the AH was not significantly different from the CON ($p<0.05$). A previous study reported that consuming a diet rich in organic sulfur compounds tended to increase some amino acids levels in rats.

Table 6: The effect of dietary supplement on the amino acids contents of guinea pig.

Items (mg/dL)	CON[1]	AH	SEM	P-value
Asparagine	4.8	4.78	0.46	0.977
Threonine	25.67	26.11	1.98	0.883
Serine	15.94	14.16	0.93	0.247
Glutamic acid	30.17	24.94	2.79	0.255
Proline	18.3	16.09	0.87	0.145
Glycine	46.31	61.5	10.21	0.352
Alanine	29.13	23.77	2.48	0.202
Cysteine	13.62	16.94	1.02	0.835
Valine	38.14	36.61	2.68	0.706
Methionine	6.23	5.82	0.66	0.683
Isoleucine	17.42	17.21	1.28	0.912
Leucine	28.86	27.62	1.78	0.648
Tyrosine	6.43	6.42	0.34	0.985
Phenylalanine	12.67	12.92	1.02	0.867
Lysine	19.6	20.56	1.32	0.634
Arginine	13.42	13.26	1.74	0.951

[1]Con: Guinea pigs fed the control diet; AH: Guinea pigs fed control diet including 1% *Allium hookeri* root powder; SEM: Standard Error of Means. The statistically different ($p<0.05$) is written as $P<0.001^{***}$; $P<0.01^{**}$; $P<0.05^{*}$ (n=5).

Conclusion

This study was performed to investigate the effect of supplementing *Allium hookeri* root powder on the body fat deposition and biochemical parameters were examined in guinea pigs. As results, the guinea pigs feed *Allium hookeri* root powder showed decreased in adipose tissue weights. Also, the feeding *Allium hookeri* root powder showed lowering triglyceride, total cholesterol, LDL-cholesterol and total SFA levels in serum. Consequently, feeding *Allium hookeri* root powder to guinea pigs may help to decrease fat accumulation, and to improve blood hyperlipidemia.

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